



TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

MONOCHLOROACETIC ACID

(CAS NO. 79-11-8)

IN F344/N RATS AND B6C3F₁ MICE

(GAVAGE STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF MONOCHLOROACETIC ACID
(CAS NO. 79-11-8)
IN F344/N RATS AND B6C3F₁ MICE
(GAVAGE STUDIES)

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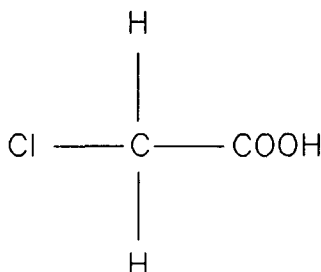
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CONTENTS

ABSTRACT	5
EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY	8
PEER REVIEW PANEL	9
SUMMARY OF PEER REVIEW COMMENTS	10
INTRODUCTION	11
MATERIALS AND METHODS	15
RESULTS	25
DISCUSSION AND CONCLUSIONS	47
REFERENCES	51
APPENDIX A Summary of Lesions in Male Rats in the 2-Year Gavage Study of Monochloroacetic Acid	55
APPENDIX B Summary of Lesions in Female Rats in the 2-Year Gavage Study of Monochloroacetic Acid	85
APPENDIX C Summary of Lesions in Male Mice in the 2-Year Gavage Study of Monochloroacetic Acid	113
APPENDIX D Summary of Lesions in Female Mice in the 2-Year Gavage Study of Monochloroacetic Acid	149
APPENDIX E Genetic Toxicology	187
APPENDIX F Organ Weights and Organ-Weight-to-Body-Weight Ratios	199
APPENDIX G Hematology and Clinical Chemistry Results	213
APPENDIX H Chemical Characterization and Dose Formulation Studies	227
APPENDIX I Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration	237
APPENDIX J Sentinel Animal Program	243
APPENDIX K Aconitase Inhibition Studies in Liver and Heart Samples	247

ABSTRACT



MONOCHLOROACETIC ACID

CAS No. 79-11-8

Chemical Formula: $\text{C}_2\text{H}_3\text{ClO}_2$ Molecular Weight: 94.5

Synonyms: Chloroacetic acid, α -chloroacetic acid, chloroethanoic acid

Monochloroacetic acid, a colorless crystalline material, is used as a postemergence contact herbicide and as an intermediate in the synthesis of other organic compounds. Toxicology and carcinogenicity studies were conducted by administering monochloroacetic acid (99% pure) in deionized water by gavage to groups of F344/N rats and B6C3F₁ mice of each sex once daily, 5 days per week for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma L5178Y cells, Chinese hamster ovary cells, and *Drosophila melanogaster*.

16-Day Studies

Groups of five rats of each sex received 0, 7.5, 15, 30, 60, or 120 mg monochloroacetic acid/kg body weight. Doses administered to mice were 0, 15, 30, 60, 120, or 240 mg/kg to groups of five males and 0, 30, 60, 120, 240, or 480 mg/kg to groups of five females. One of five male rats given 120 mg/kg died during the studies. Clear nasal discharge, lacrimation, or both, were observed in all groups of male and female rats receiving monochloroacetic acid. No compound-related gross lesions were observed in rats. All male mice given 240 mg/kg and all females given 240 or 480 mg/kg died during the studies. Hypoactivity, piloerection, ataxia, and lacrimation were observed in mice given 240 or 480 mg/kg. No

compound-related gross lesions were observed in mice at necropsy.

13-Week Studies

Groups of 20 rats of each sex received 0, 30, 60, 90, 120, or 150 mg/kg monochloroacetic acid, and groups of 20 mice of each sex received doses of 0, 25, 50, 100, 150, or 200 mg/kg. Three to five animals in each dose group were killed at weeks 4 and 8 for the evaluation of hematology parameters. Compound-related deaths occurred in rats in the three highest dose groups (all males given 120 or 150 mg/kg, 9/10 males given 90 mg/kg, and all females given 90 to 150 mg/kg) and in mice given 200 mg/kg (all males and 2/10 females). Final mean body weights of surviving rats and mice receiving monochloroacetic acid were similar to those of controls. In rats, dose-related increases in blood urea nitrogen, alanine aminotransferase, and aspartate aminotransferase levels were observed, and relative liver and kidney weights were elevated. There were no compound-related changes in the various hematologic or clinical pathology parameters in mice. A dose-related increase in the incidence and severity of cardiomyopathy was observed in male and female rats receiving monochloroacetic acid, and hepatocellular cytoplasmic vacuolization was observed in the high-dose mice that died during the studies.

2-Year Studies

Based on the mortality and compound-related histopathologic lesions observed in the 13-week studies, doses selected for the 2-year studies of monochloroacetic acid were 0, 15, or 30 mg/kg, administered to groups of 70 rats of each sex, and 0, 50, or 100 mg/kg, administered to groups of 60 mice of each sex. Interim evaluations were conducted on 10 rats per dose group after 6 months of treatment with monochloroacetic acid and on seven rats per dose group after 15 months of treatment.

Body Weight and Survival in the 2-Year Studies

Mean body weights of low- and high-dose female and low-dose male rats receiving monochloroacetic acid were within 10% of those of controls throughout the studies; however, after week 30, the mean body weights of high-dose male rats were 4% to 8% less than those of controls. In mice, the mean body weights of dosed males were similar to controls, but those of low- and high-dose females were 6% to 10% less than control values after week 52. Survival of high-dose male and dosed female rats and high-dose male mice was significantly lower than that of controls (male rats: control, 27/53; low-dose, 21/53; high-dose, 16/53; female rats: 37/53; 19/53; 26/53; male mice: 46/60; 39/60; 21/60; female mice: 42/60; 40/60; 44/60).

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies

There was no compound-related increase in the incidence of neoplasms or nonneoplastic lesions in rats given monochloroacetic acid for 2 years. The incidence of uterine stromal polyps in low- and high-dose female rats was slightly higher than that in controls (2/60; 7/57; 10/60). However, the incidence in the controls was unusually low, and those in the dosed groups were well within the range for NTP historical controls (mean: 21%, range: 10%-38%). Further, because the only malignant stromal neoplasm occurred in a control animal,

the polyps were not considered to be related to the administration of monochloroacetic acid. Similarly, there was no monochloroacetic acid-related increase in the incidence of neoplasms in male or female mice, and malignant lymphoma occurred with a significant negative trend in dosed female mice. Increases in the incidence of inflammation of the mucosa of the nasal passages, respiratory epithelial metaplasia of the olfactory epithelium of the nose, and focal squamous cell hyperplasia of the forestomach occurred in dosed male and female mice.

Genetic Toxicology

Monochloroacetic acid was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98, with or without exogenous metabolic activation (S9). It induced trifluorothymidine resistance in L5178Y cells in the absence of S9 and induced sister chromatid exchanges without S9 in Chinese hamster ovary cells. Monochloroacetic acid did not induce a significant increase in chromosomal aberrations in Chinese hamster ovary cells, with or without S9. Monochloroacetic acid administered in feed was negative for the induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster*; however, when it was administered by injection, the results were equivocal.

Conclusions

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** for monochloroacetic acid in male or female F344/N rats given 15 or 30 mg/kg. There was *no evidence of carcinogenic activity* for monochloroacetic acid in male or female B6C3F₁ mice given 50 or 100 mg/kg.

Monochloroacetic acid administration was associated with inflammatory lesions of the nasal mucosa, metaplasia of the olfactory epithelium, and squamous cell hyperplasia of the forestomach in male and female mice.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of peer review comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Monochloroacetic Acid

Variable	Male F344/N Rats	Female F344/N Rats	Male B6C3F ₁ Mice	Female B6C3F ₁ Mice
Doses	0, 15, or 30 mg/kg in deionized water by gavage 5 days per week	0, 15, or 30 mg/kg in deionized water by gavage 5 days per week	0, 50, or 100 mg/kg in deionized water by gavage 5 days per week	0, 50, or 100 mg/kg in deionized water by gavage 5 days per week
Body weights	Dosed groups similar to controls	Dosed groups similar to controls	Dosed groups similar to controls	Dosed groups lower than controls
2-Year survival rates	27/53, 21/53, 16/53	37/53, 19/53, 26/53	46/60, 39/60, 21/60	42/60, 40/60, 44/60
Nonneoplastic effects	None	None	Inflammation of nasal mucosa (3/60, 7/59, 24/60); Metaplasia of olfactory epithelium (0/60, 3/59, 2/60) Squamous hyperplasia of the forestomach (5/60, 2/60, 13/60)	Inflammation of nasal mucosa (5/60, 15/60, 31/60); Metaplasia of olfactory epithelium (2/60, 5/60, 17/60); Squamous hyperplasia of the forestomach (5/60, 8/59, 15/60)
Neoplastic effects	None	None	None	None
Uncertain findings	None	None	None	Malignant lymphoma (29/60, 18/60, 13/60)
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
<i>Salmonella typhimurium</i> gene mutation:		Negative with or without S9 in strains TA100, TA1535, TA1537, or TA98		
L5178Y mouse lymphoma gene mutation:		Positive without S9		
Sister chromatid exchanges				
Chinese hamster ovary cells <i>in vitro</i> :		Positive without S9; negative with S9		
Chromosomal aberrations				
Chinese hamster ovary cells <i>in vitro</i> :		Negative with or without S9		
Sex-linked recessive lethal mutations				
<i>Drosophila melanogaster</i> male germ cells:		Negative when administered in feed Equivocal when administered by injection		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the NTP draft Technical Report on monochloroacetic acid on November 20, 1990 are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS

On November 20, 1990, the draft Technical Report on the toxicology and carcinogenesis studies of monochloroacetic acid received public review by the National Toxicology Program (NTP) Board of Scientific Counselors' Technical Reports Review Committee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC.

Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of monochloroacetic acid by discussing the uses of this compound and the rationale for its study, describing the experimental design, reporting on survival and body weight effects, and commenting on nonneoplastic lesions that were observed. The conclusions were *no evidence of carcinogenic activity* of monochloroacetic acid for male or female F344/N rats or B6C3F₁ mice.

Dr. Davis, the first principal reviewer, agreed with the conclusions. He commented that a maximum tolerated dose may not have been reached for female mice because the difference between control and high-dose group final mean body weights was only 6%. He further noted that from week 53 to week 103 the difference in mean body weights between the two groups was only 9%. Dr. S.L. Eustis, NIEHS, explained that a consistent decrement in body weight over a long period of time usually represents a toxic effect, even if the decrement is less than 10%, as in these studies. Dr. J.K. Haseman, NIEHS, added that the nonneoplastic lesions of the nasal cavity and forestomach observed

in this study suggested that a maximum tolerated dose had been achieved.

Dr. Longnecker, the second principal reviewer, agreed with the conclusions.

Dr. Ashby, the third principal reviewer, agreed with the conclusions. He noted that the genetic toxicity profile continued a trend established in earlier studies, namely, no structural alert, no mutagenicity in *Salmonella*, and no clastogenicity in Chinese hamster ovary cells, but induction of mutations in L5178Y cells and of sister chromatid exchanges in Chinese hamster ovary cells. He added that this pattern confirmed that the latter two protocols were not correlated with *in vivo* carcinogenicity.

There was some discussion about the forestomach lesions in mice and, in particular, about the increased incidence of squamous cell papillomas in females. The discussion of the papillomas centered on whether the significant increase in incidence of hyperplasia in the high-dose group reflected a preneoplastic effect or focal irritation due to gavage with an irritating substance. The NTP staff supported the latter position.

Dr. Davis moved that the Technical Report on monochloroacetic acid be accepted with the revisions discussed and the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity*. Dr. Longnecker seconded the motion, which was accepted unanimously with 11 votes.